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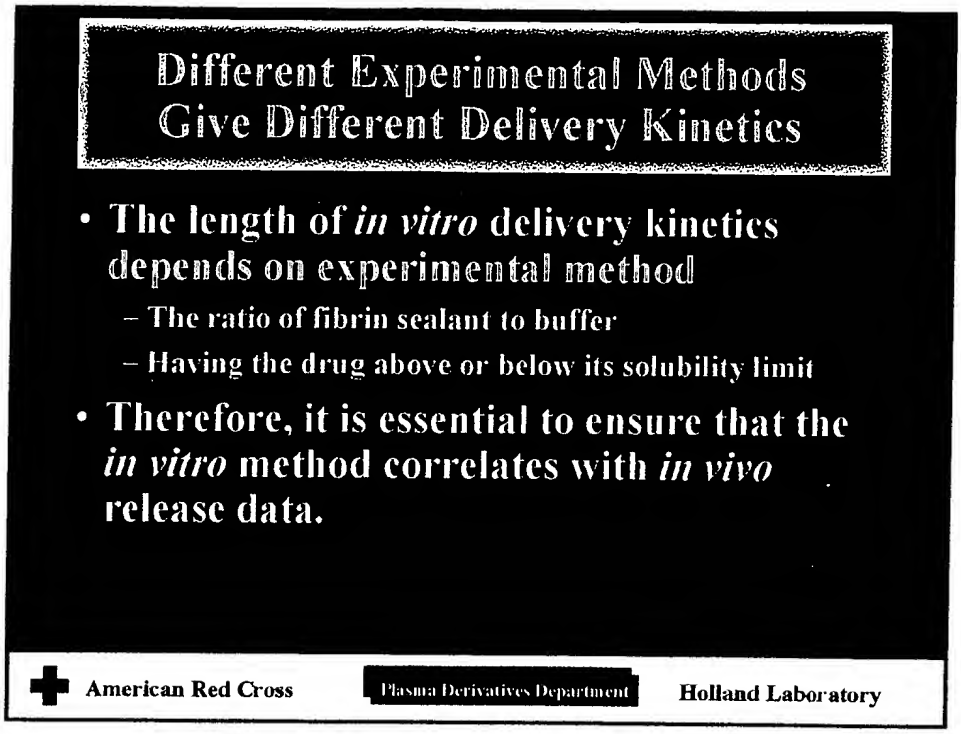
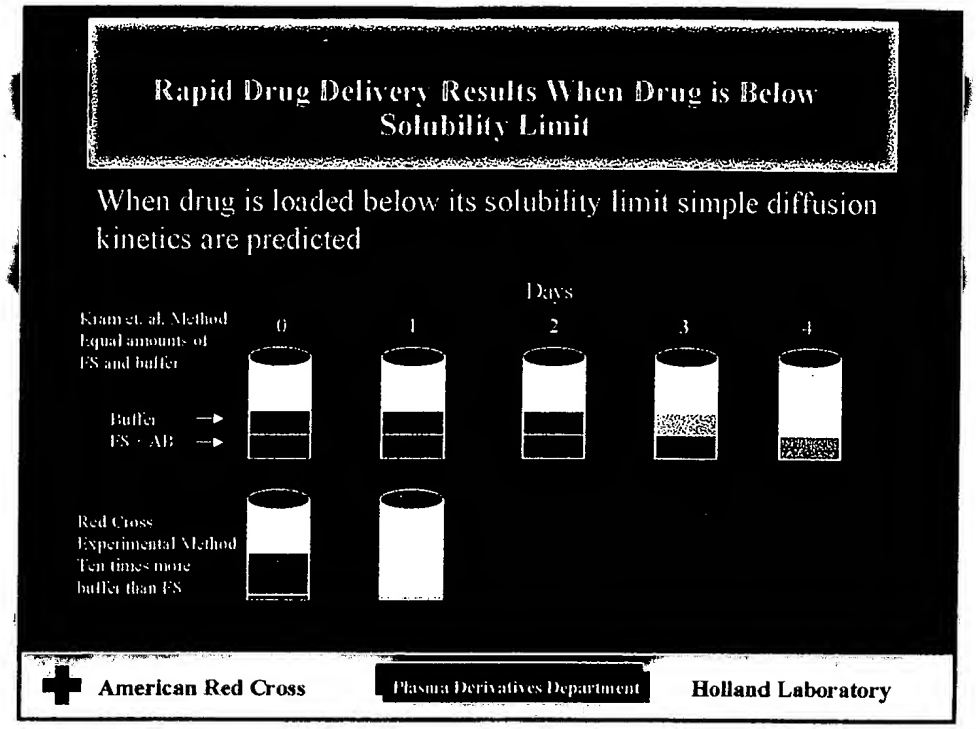


Slide 1.

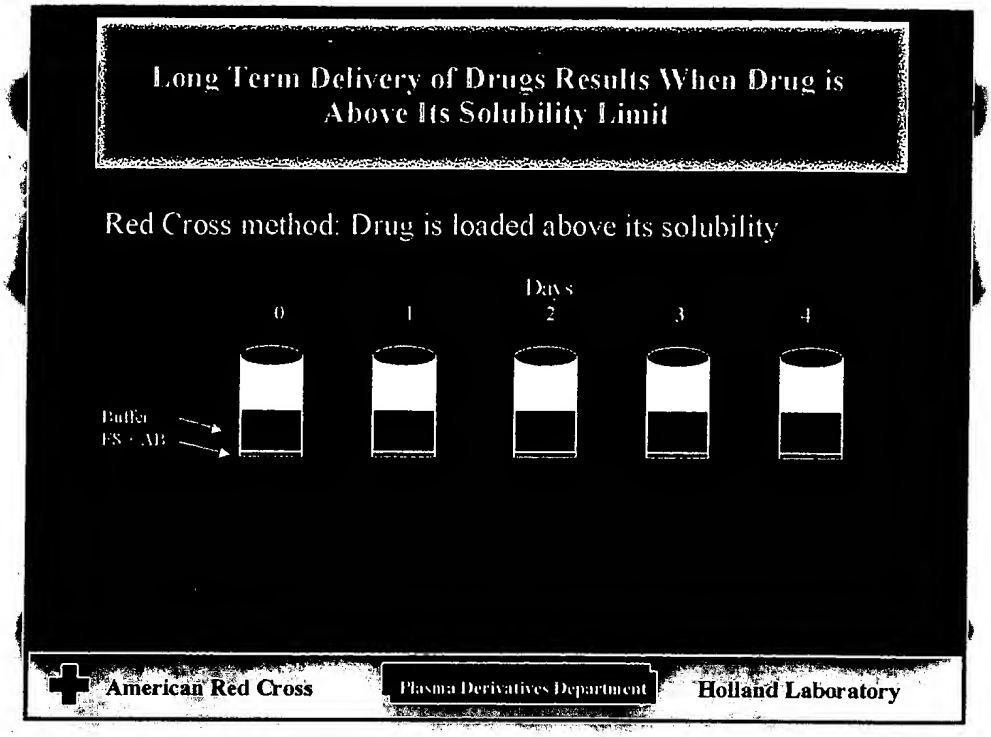
Slide 1 is an overview of the presentation. It develops the key points that were examined in the presentation. The first key point, which varied from our data and that of the cited literature, was the ratio between the drug supplemented fibrin sealant and the buffer. This ratio will change the drug delivery kinetics when the drug is loaded below its solubility limit. The second key point is having the drug incorporated above its solubility limit. Only our system places the drug above its solubility limits and this is claimed as part of the claim set. The final key point of the slide is that there must be a correlation between the *in vitro* delivery data and the *in vivo* delivery data. The American Red Cross has made this correlation, others have not. The rest of the slides will examine these key points in detail.



Slide 2.

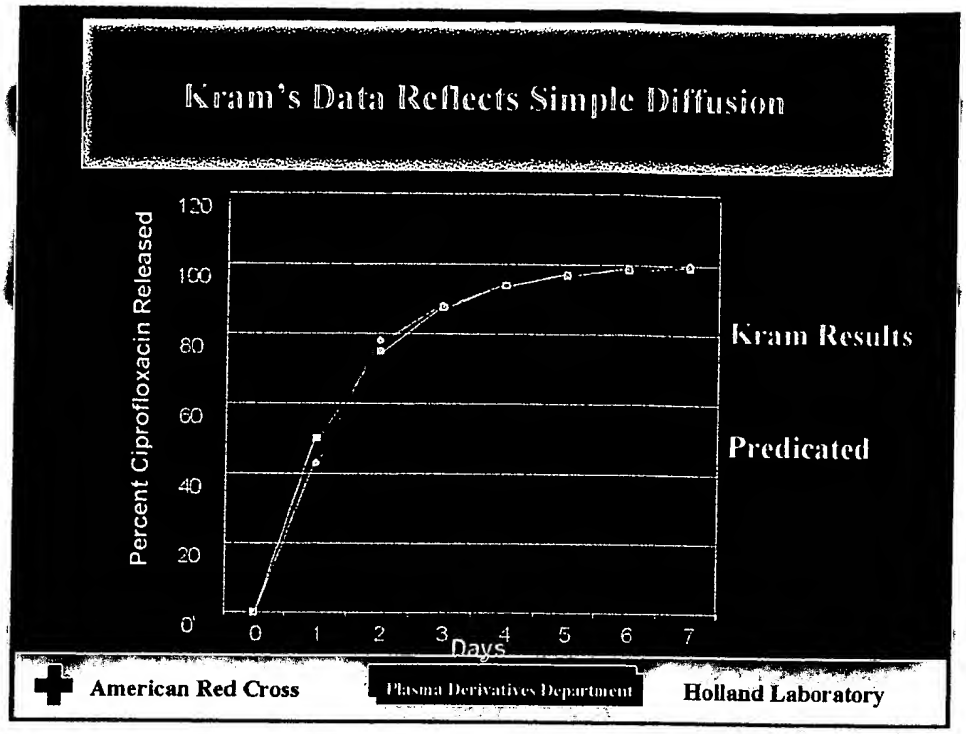
This slide examines the rapid drug delivery when the drug is below its solubility limit. The slide compares Kram et. al. delivery methods to the American Red Cross methods. Kram's data was chosen as the comparison because their data showed the longest delivery kinetics from all the literature cited. The reason for the Kram's longer delivery kinetics is in their experimental methods. Kram uses a 1:1 ratio between the fibrin sealant and the buffer the drug diffuses into. The American Red Cross experimental methods use a 1:10 ratio of fibrin sealant to buffer. Both methods change the buffer once a day.

What do the different experimental methods mean for a simple diffusion model of the drug from the fibrin sealant? Using the Kram method, 50% of the drug will diffuse into the buffer the first day since it is a 1:1 dilution. By the second day, another 50% of the remaining drug (75% of the total starting drug) will diffuse out of the fibrin sealant. The American Red Cross method of a 1:10 ratio will have 90% of the drug diffuse out the first day when the drug is placed into the fibrin sealant below its solubility limit. By the second day, only 1% of the drug will remain in the fibrin sealant by the simple diffusion model using the American Red Cross method.



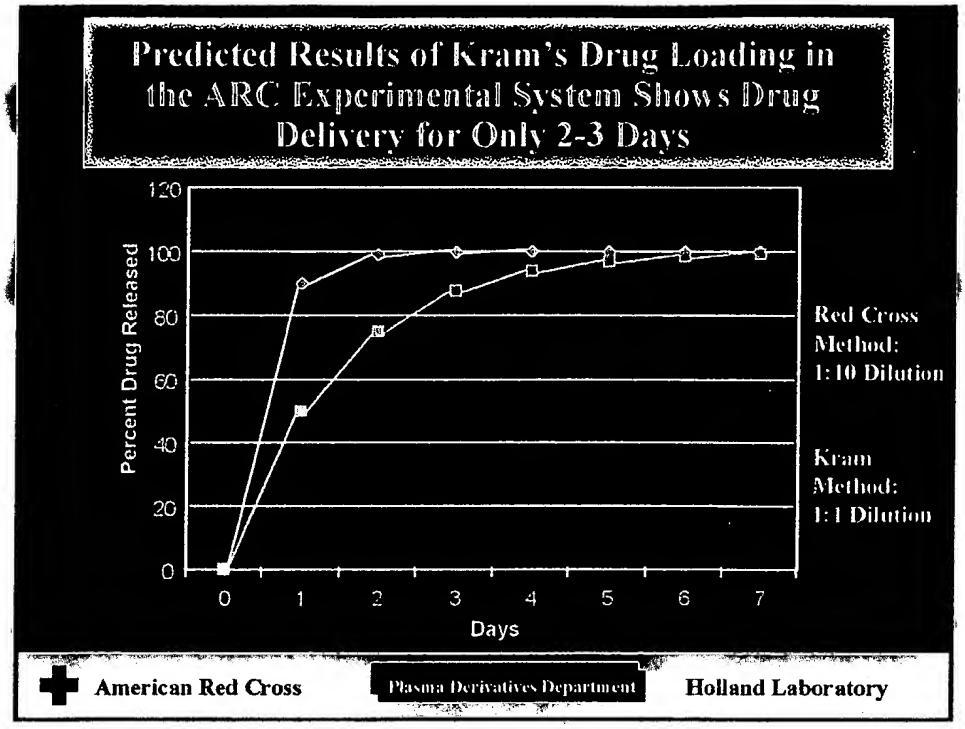
Slide 3.

This slide shows a representative cartoon when the drug is loaded above its solubility limit using the American Red Cross method. Since it is above its solubility limit one does not loose 90% of the drug the first day, but only a small fraction of the drug is released per day. This is how the American Red Cross is able to get sustained release. Real drug delivery data will be presented in later slides.



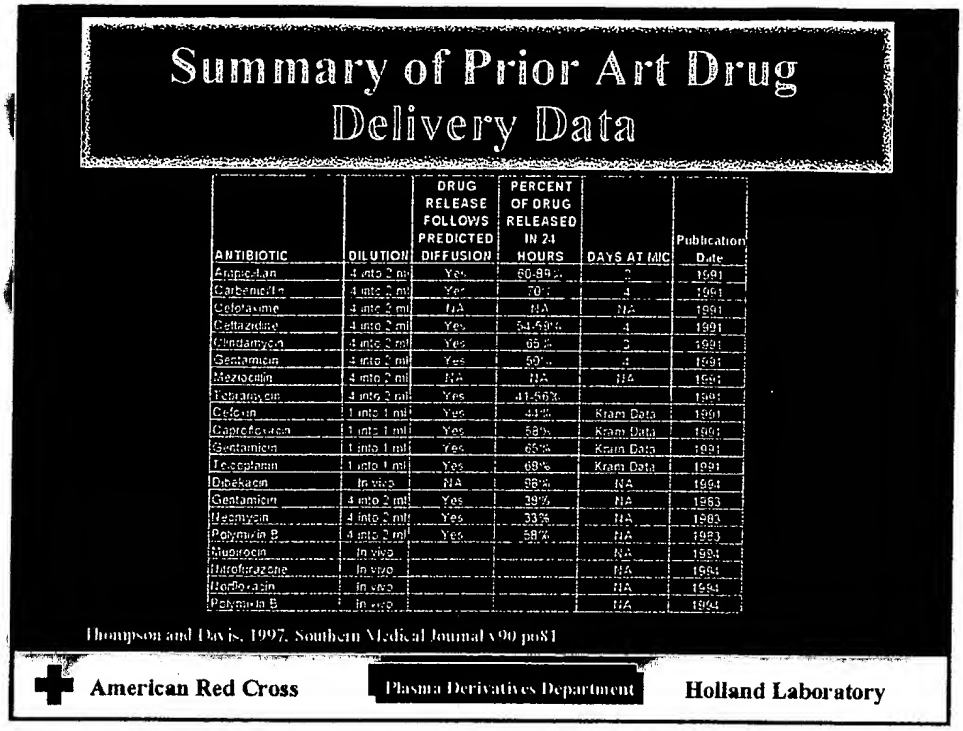
Slide 4.

This slide shows a graph of Kram's delivery data for ciprofloxacin vs the mathematically predicted delivery kinetics using Kram's 1:1 experimental methods ratio of fibrin sealant to buffer. Kram's experimental data follows the same curve as the predicted by the 1:1 serial dilution curve. Therefore , Kram's data follows simple diffusion kinetics. The only reason Kram has 7 days delivery is the 1:1 dilution ratio of his experimental methods.



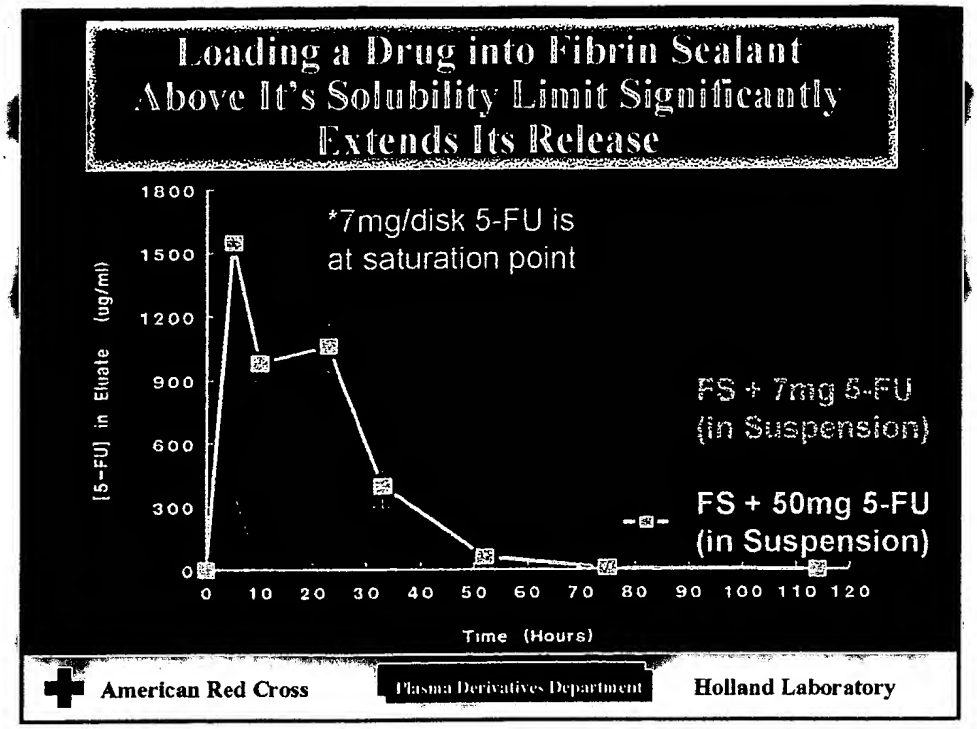
Slide 5.

This slide shows the mathematically predicted delivery kinetics of drugs loaded below the solubility limit using the American Red Cross 1:10 dilution method and Kram's 1:1 dilution method. Using the American Red Cross method, 99% of the drug is released after two days. It is not until day 7 using the Kram method that 99% of the drug is released. In some cases, Kram's antibiotic concentrations were greater than 100 fold over their minimal inhibitory concentrations. Therefore, after seven days Kram still have enough antibiotic to claim activity. If Kram used the American Red Cross ratios, there would be no activity after 3 days. In the Kram case, simple diffusion rules.



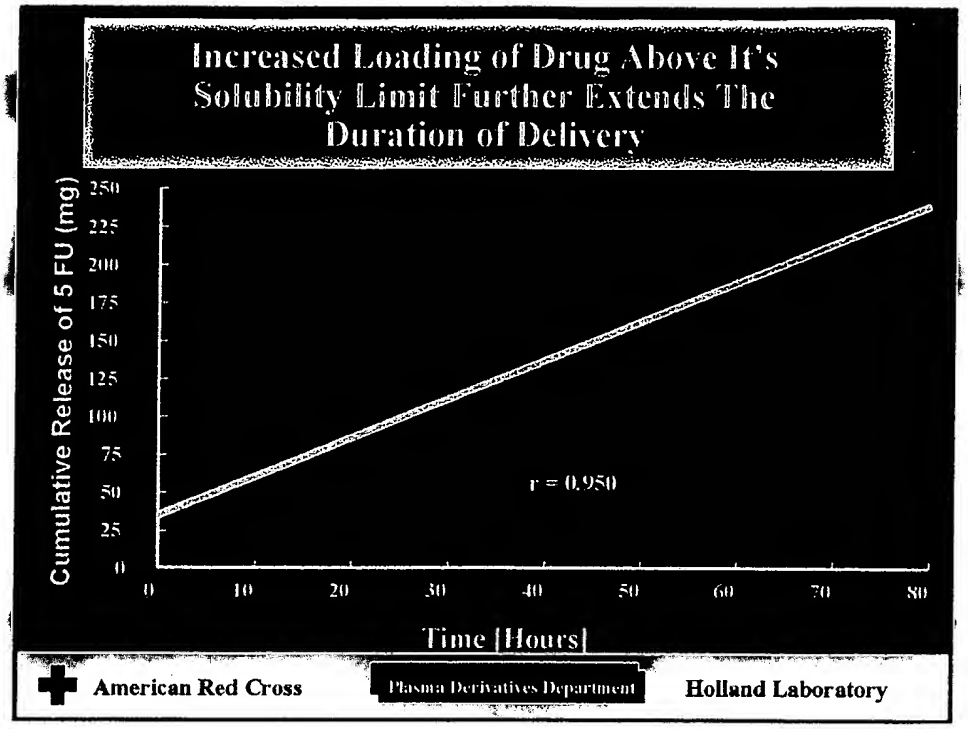
Slide 6

This slide is a summary slide of other literature from Thompson and Davis claiming sustained release of antibiotics (Thompson and Davis, 1997, Southern Medical Journal v90 p681). These authors claim long term delivery kinetics, but none of them use the 1:10 ratio method that the American Red Cross uses. Kram's data showed the longest delivery kinetics. Kram was the only one to use a 1:1 ratio. All the others used a 1:5 dilution. All other data in the table follows a simple diffusion model of kinetics for their dilutions. This relates to the first key point in our introductory slide (slide 1). The delivery kinetics depends on the experimental design. The American Red Cross uses a 1:10 ratio. That is the most stringent experimental method and the American Red Cross gets sustained release only when the drug is above the solubility limit.



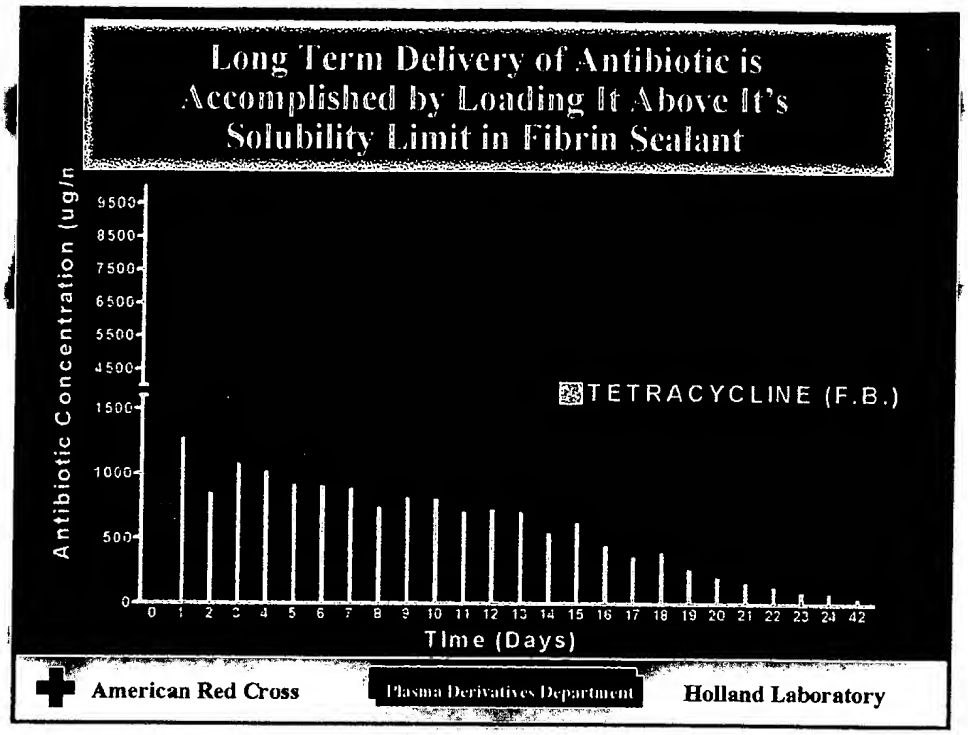
Slide 7

This slide shows the release kinetics of 5-Flourouracil (5-FU) when the drug is placed above its solubility limit. If the release kinetics of 5-FU followed the simple dilution of the literature cited against us then 90% of the initial 50mg in 2 ml or 45 mg of 5-FU would have diffused out at the first time point, but only 1.6 mg/ml was released. Drug release is seen for 30 hours, four time points. This shows that loading above the solubility limit does not follow the simple diffusion kinetics into the dilution ratio. Thus loading drugs above the their solubility point extends the duration the drug delivery. The second key point in the introductory slide.



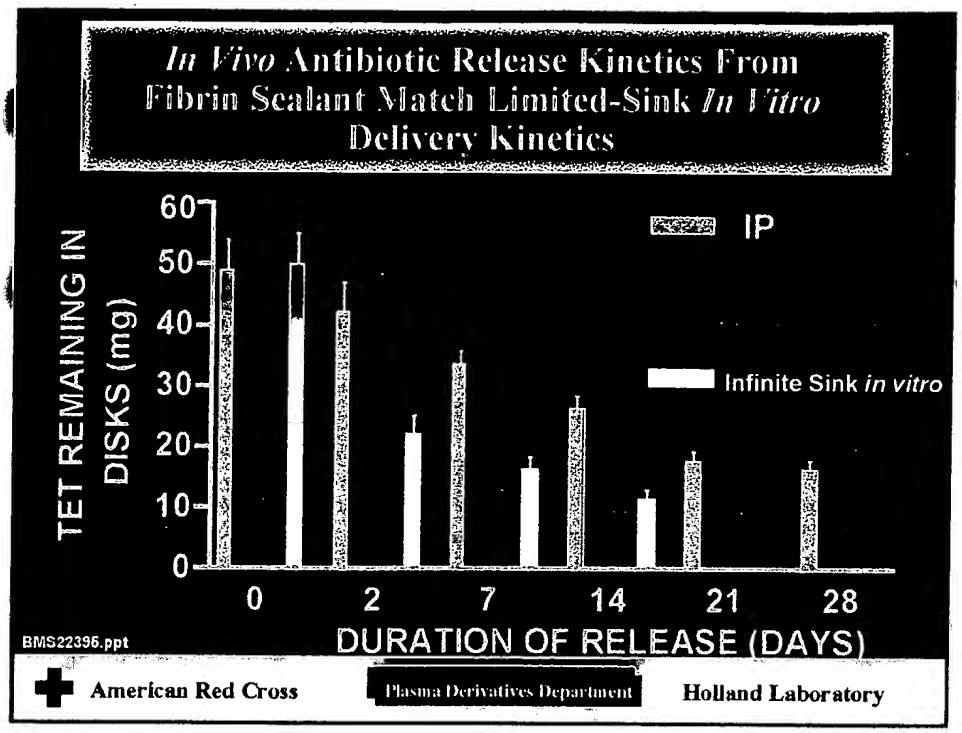
Slide 8

This slide shows the release of 250 mg of 5-FU instead of 50 mg shown in the previous slide. This data is plotted differently. The previous slide is the amount released per day and this slide shows the cumulative release. By the addition of more material the delivery kinetics was extended to 80 hours. One also sees that there is a linear relationship with the delivery of the drug indicating a constant uniform delivery of the drug.



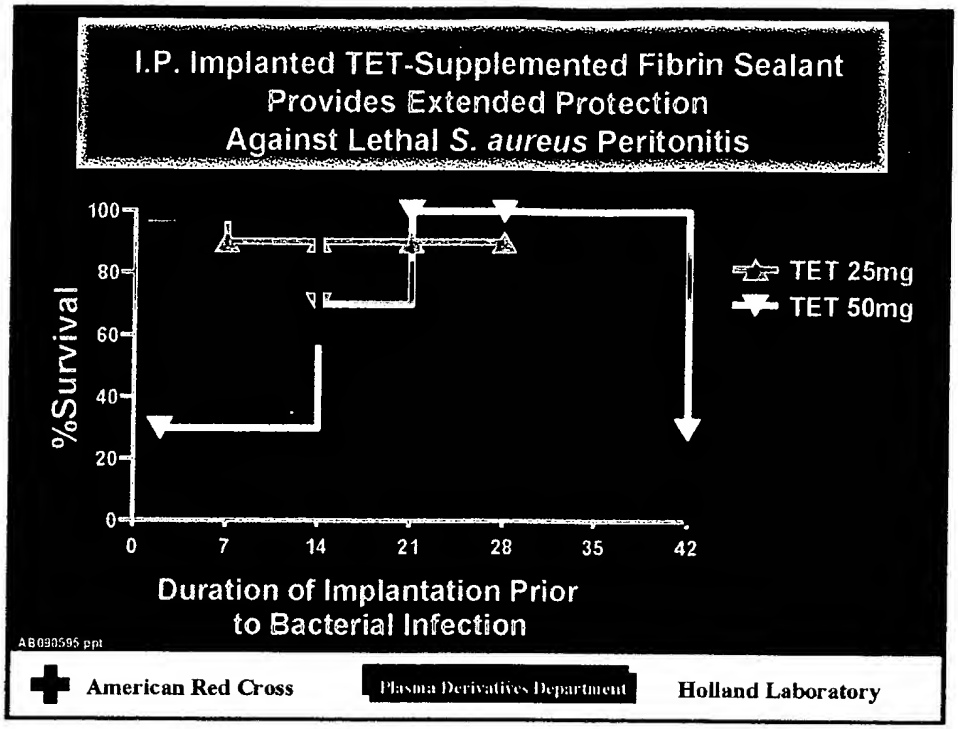
Slide 9

This slide shows three examples of the delivery kinetics of different drugs loaded above their solubility limits. The data shows that the American Red Cross was able to deliver a therapeutic dose of drugs for weeks.



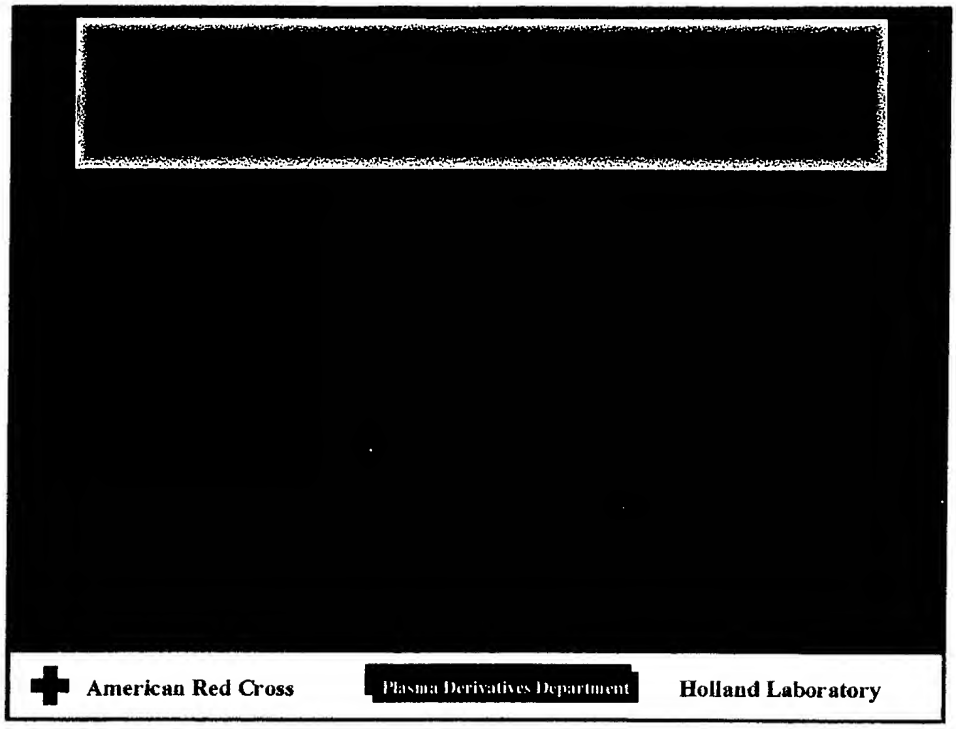
Slide 10

Up to this point, the data shows sustained *in vitro* drug delivery. The *in vitro* methods use a stringent 1:10 dilution, but why was a 1:10 dilution selected? Why not a higher dilution? To answer this question, the American Red Cross compared the *in vitro* data with *in vivo* delivery kinetics. This slide shows the *in vivo* delivery kinetics with *in vitro* delivery data. The delivery kinetics of an *in vitro* 1:10 dilution (limited sink) and a 1:225 dilution (pseudo-infinite sink which will be referred here as a infinite sink) drug delivery model was compared to an i.p. or subQ injection of tetracycline loaded above its solubility into mice. The data shows that a 1:10 *in vitro* dilution model mimics *in vivo* events. The 1:225 infinite sink dilution released the drug faster than *in vivo*. That is why the previous experiments were done at a 1:10 dilution.



Slide 11

So far we have shown that a 1:10 dilution mimics the *in vivo* model and loading above the solubility results in sustained drug release. What remains to be demonstrated is efficacy. This slide shows how an i.p. injection of tetracycline into mice is able to protect the mice from a lethal injection of S. aureus. At time zero, different concentration of tetracycline supplemented fibrin sealant was injected i.p. into mice. At the indicated times, the mice were then injected with a lethal dose of S. aureus. The data shows that the higher the initial dose of tetracycline the longer the drug was able to protect against S. aureus which indicates longer drug delivery. At 50 mg, protection, and therefore sustained delivery, went for as long as 42 days. Lower doses resulted in shorter durations of protection. Therefore, the American Red Cross was able to vary drug concentration to give a desired therapeutic dose regimen. Nobody has demonstrated *in vivo* long term delivery of a therapeutic dose of a drug from fibrin sealant prior to our filings. Our collaborators have and will be publishing examples of the therapeutic use of antibiotic loaded above their solubility limits in fibrin sealant for the treatment of infections in humans.



Summary (No Slide)

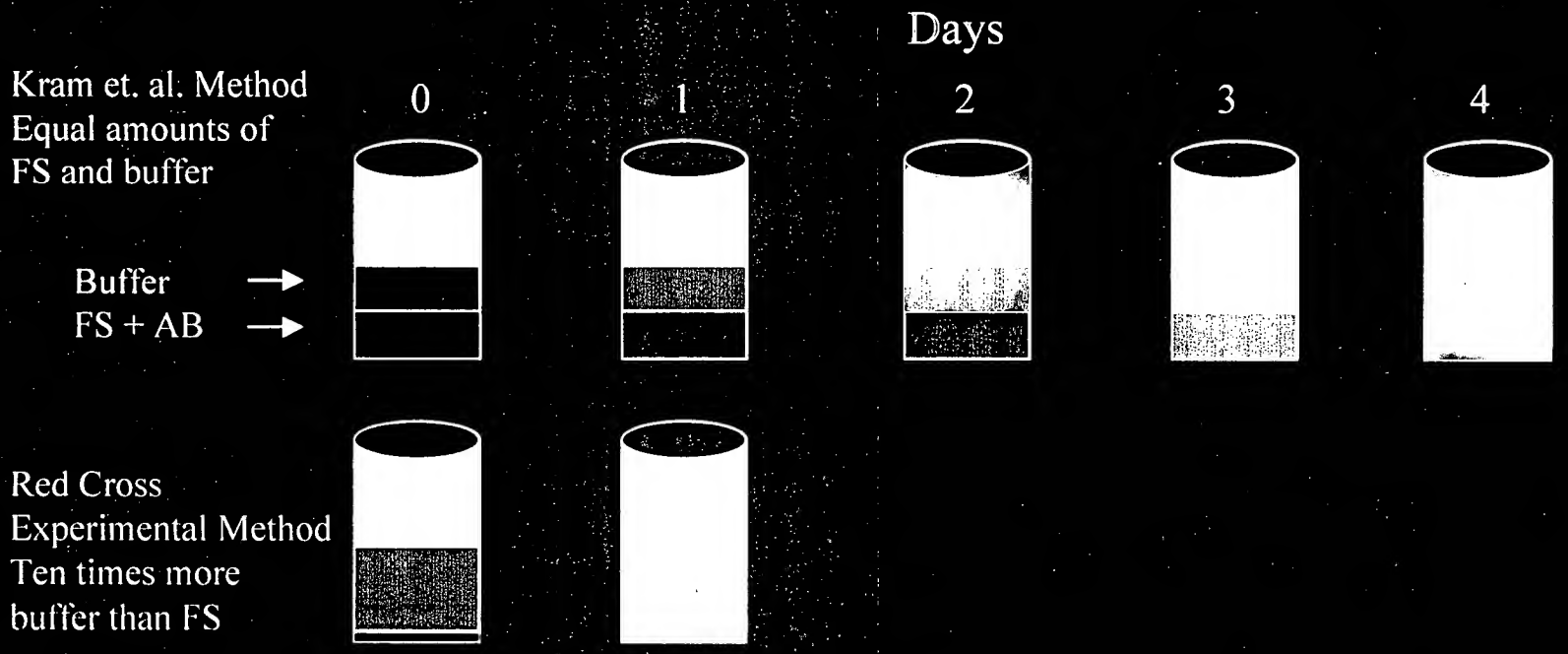
The American Red Cross has demonstrated novel formulations of drugs loaded above their solubility limits in fibrin sealant to obtain sustained drug release. The prior art cited against us does not load the drugs above their solubility limits and their claims of sustained delivery are due to their experimental methods. They use a low ratio of the volumes of fibrin sealant to buffer. What the prior art shows is simple diffusion kinetics from fibrin sealant as the buffer dilutes out the drug. The American Red Cross uses a high ratio of fibrin sealant to buffer. This ratio was selected from an experiment comparing *in vitro* and *in vivo* delivery kinetics. Finally, the American Red Cross demonstrated *in vivo* efficacy of their sustained drug delivery system.

Different Experimental Methods Give Different Delivery Kinetics

- The length of *in vitro* delivery kinetics depends on experimental method
  - The ratio of fibrin sealant to buffer
  - Having the drug above or below its solubility limit
- Therefore, it is essential to ensure that the *in vitro* method correlates with *in vivo* release data.

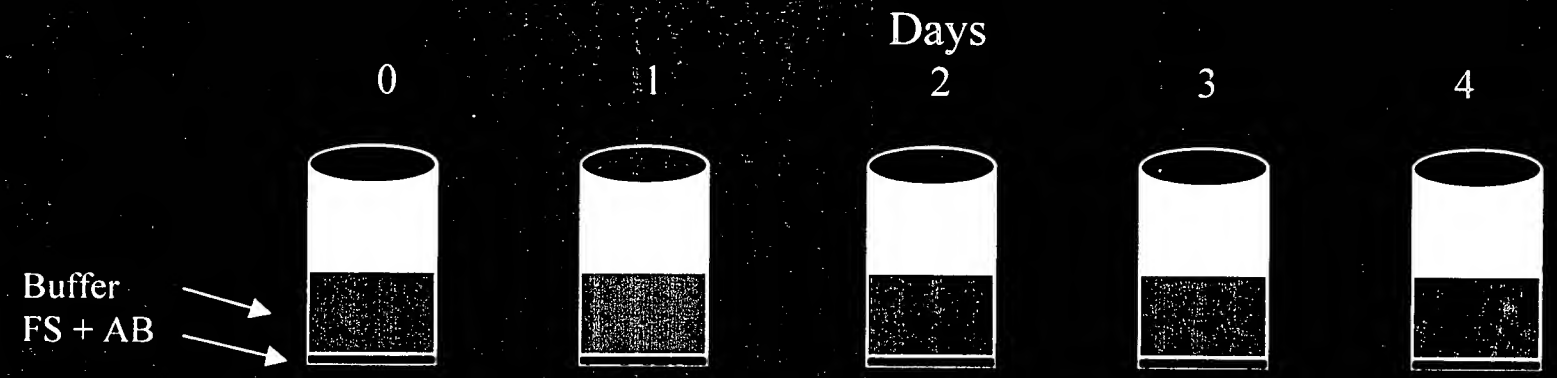
Rapid Drug Delivery Results When Drug is Below Solubility Limit

When drug is loaded below its solubility limit simple diffusion kinetics are predicted

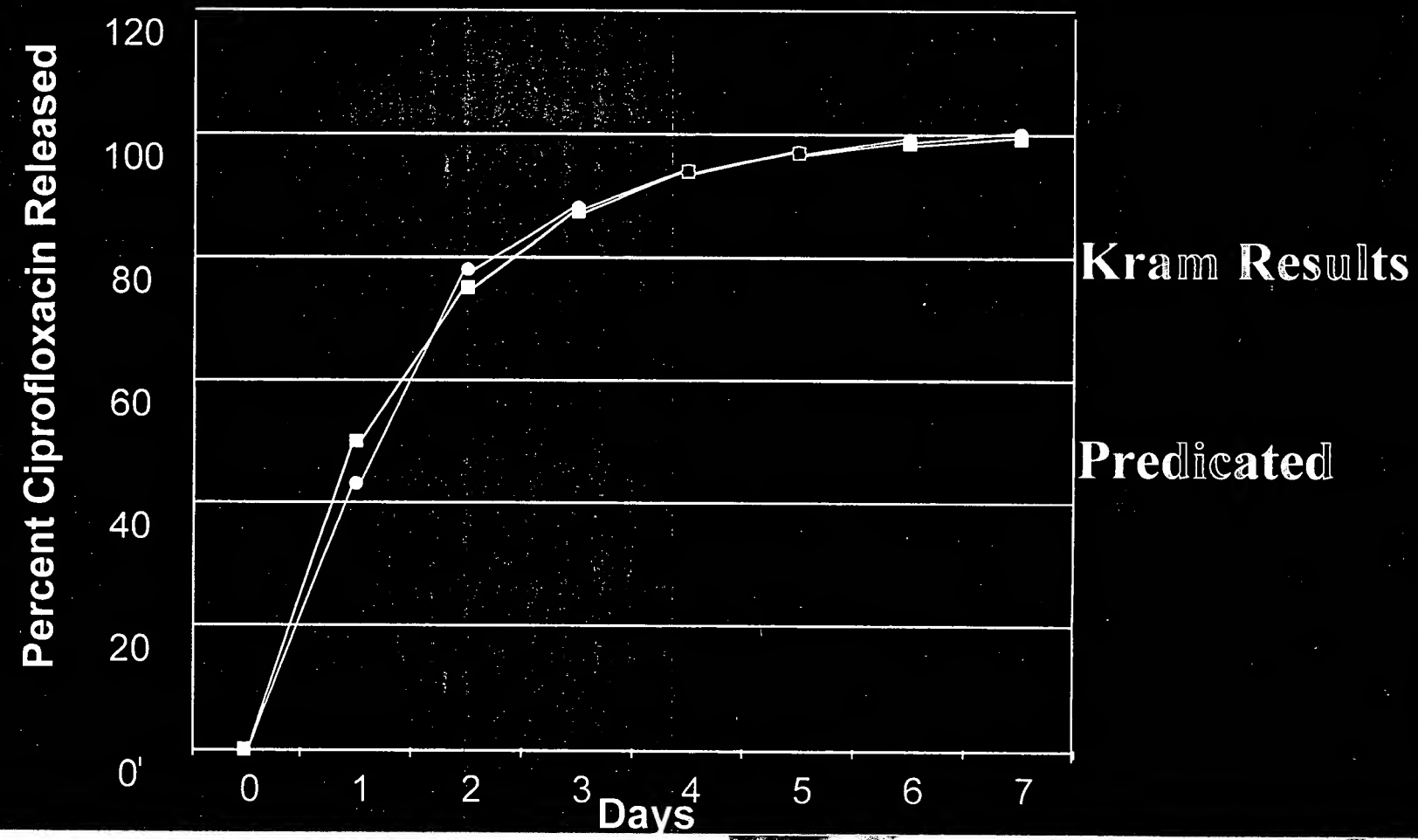


Long Term Delivery of Drugs Results When Drug is Above Its Solubility Limit

Red Cross method: Drug is loaded above its solubility



Kram's Data Reflects Simple Diffusion



American Red Cross

Plasma Derivatives Department

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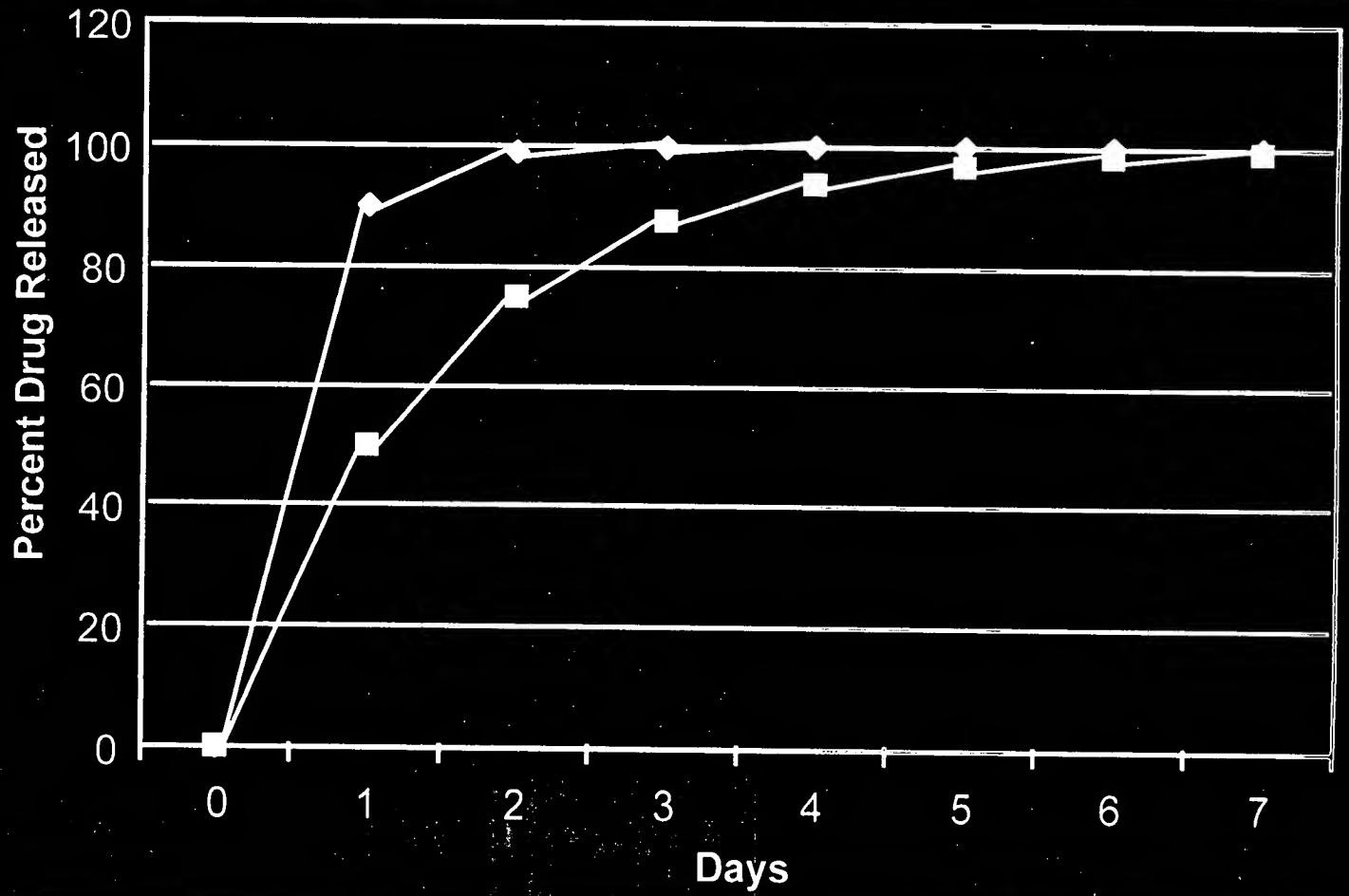
Predicted Results of Kram's Drug Loading in the ARC Experimental System Shows Drug Delivery for Only 2-3 Days



Red Cross Method: 1:10 Dilution

Kram Method: 1:1 Dilution

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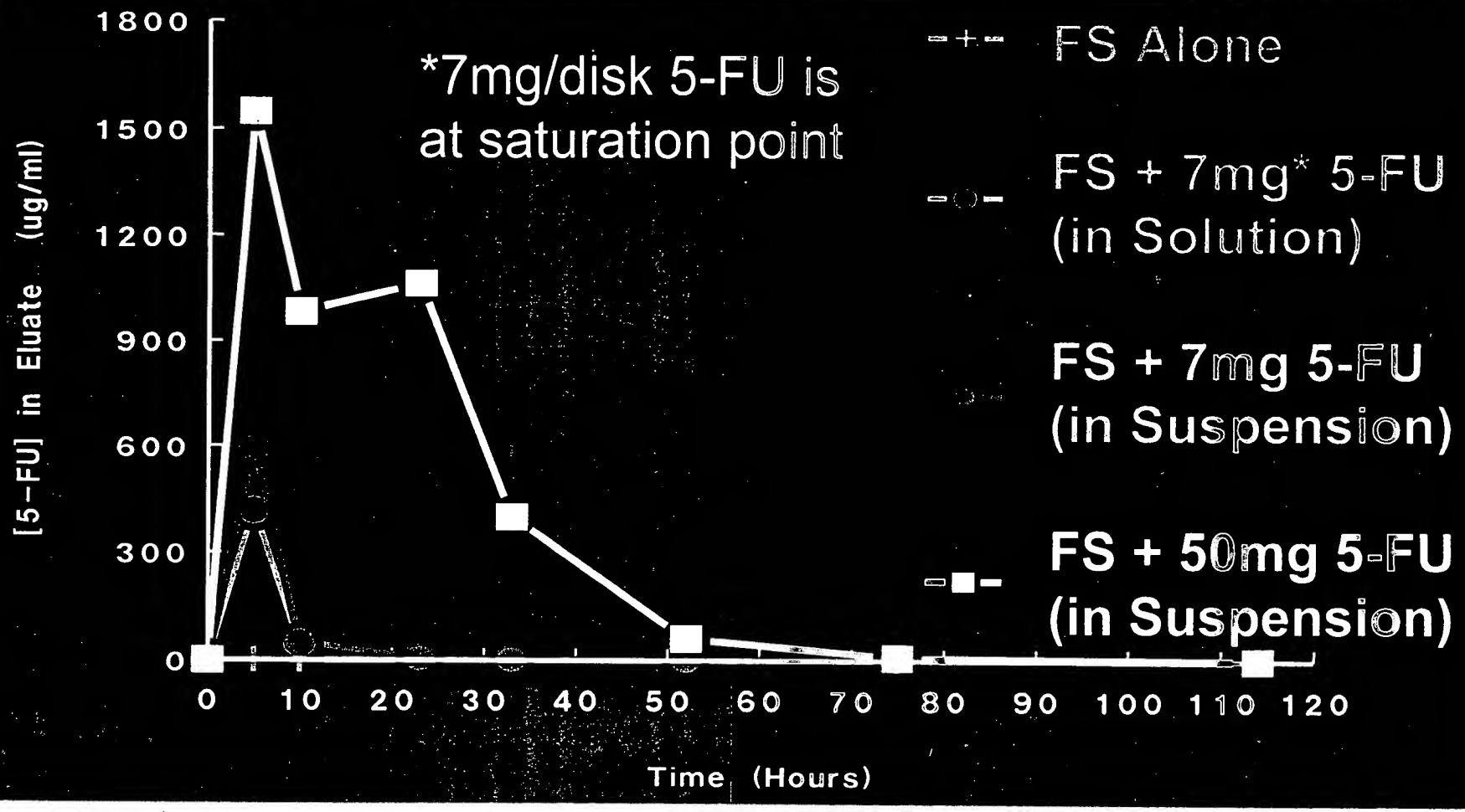
Summary of Prior Art Drug Delivery Data

ANTIBIOTIC	DILUTION	DRUG RELEASE FOLLOWS PREDICTED DIFFUSION	PERCENT OF DRUG RELEASED IN 24 HOURS	DAYS AT MIC	Publication Date
Ampicillian	.4 into 2 ml	Yes	60-89%	2	1991
Carbenicillin	.4 into 2 ml	Yes	70%	4	1991
Cefotaxime	.4 into 2 ml	NA	NA	NA	1991
Ceftazidine	.4 into 2 ml	Yes	54-59%	4	1991
Clindamycin	.4 into 2 ml	Yes	65%	3	1991
Gentamicin	.4 into 2 ml	Yes	50%	4	1991
Mezlocillin	.4 into 2 ml	NA	NA	NA	1991
Tobramycin	.4 into 2 ml	Yes	41-56%		1991
Cefoxin	1 into 1 ml	Yes	44%	Kram Data	1991
Caprofloxacin	1 into 1 ml	Yes	58%	Kram Data	1991
Gentamicin	1 into 1 ml	Yes	65%	Kram Data	1991
Teicoplanin	1 into 1 ml	Yes	68%	Kram Data	1991
Dibekacin	In vivo	NA	98%	NA	1994
Gentamicin	.4 into 2 ml	Yes	38%	NA	1983
Neomycin	.4 into 2 ml	Yes	33%	NA	1983
Polymixin B	.4 into 2 ml	Yes	58%	NA	1983
Mupirocin	In vivo			NA	1994
Nitrofurazone	In vivo			NA	1994
Norfloxacin	In vivo			NA	1994
Polymixin B	In vivo			NA	1994

Thompson and Davis, 1997, Southern Medical Journal v90 p681

Loading a Drug into Fibrin Sealant Above It's Solubility Limit Significantly Extends Its Release



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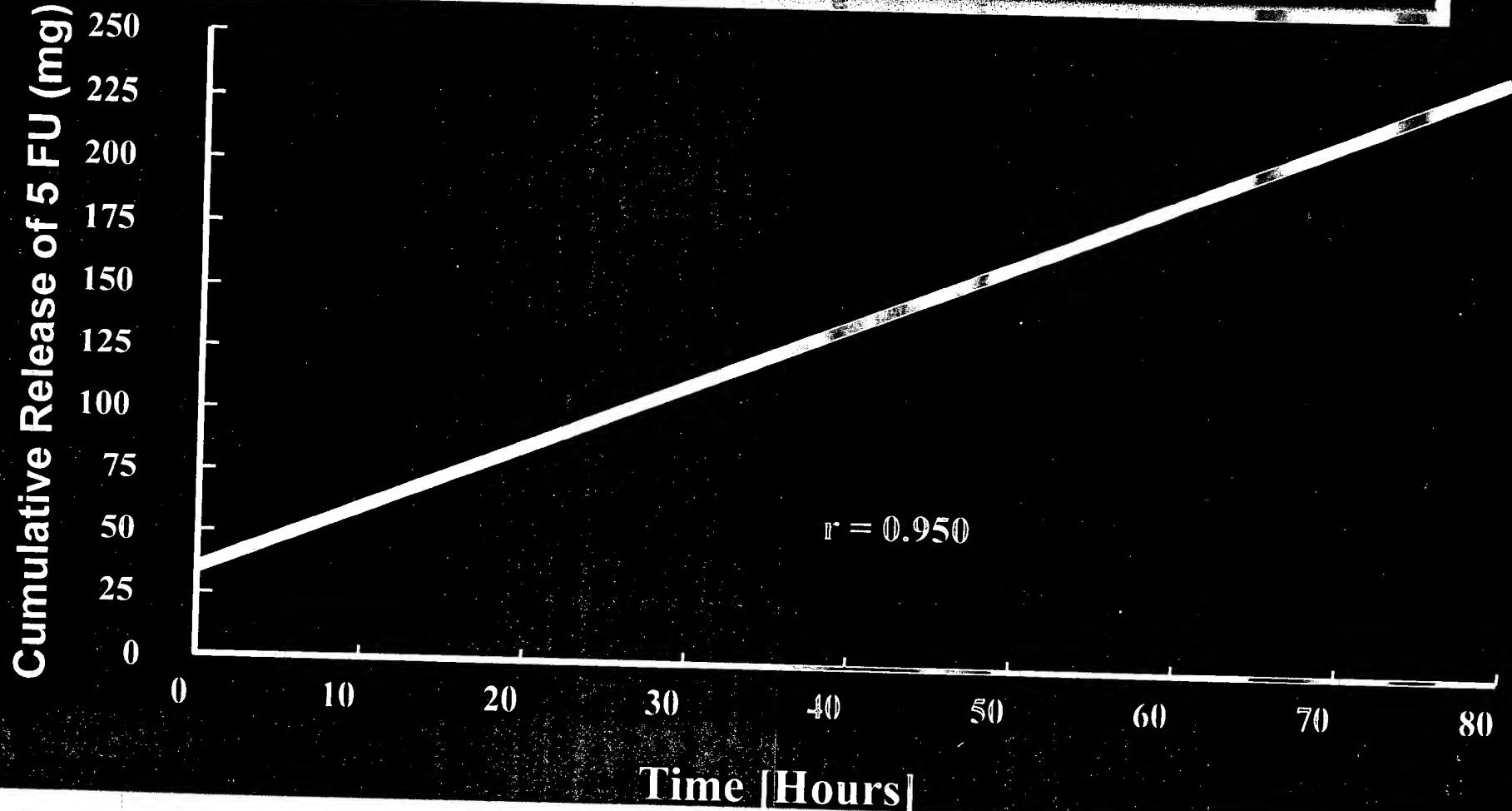
Increased Loading of Drug Above It's Solubility Limit Further Extends The Duration of Delivery



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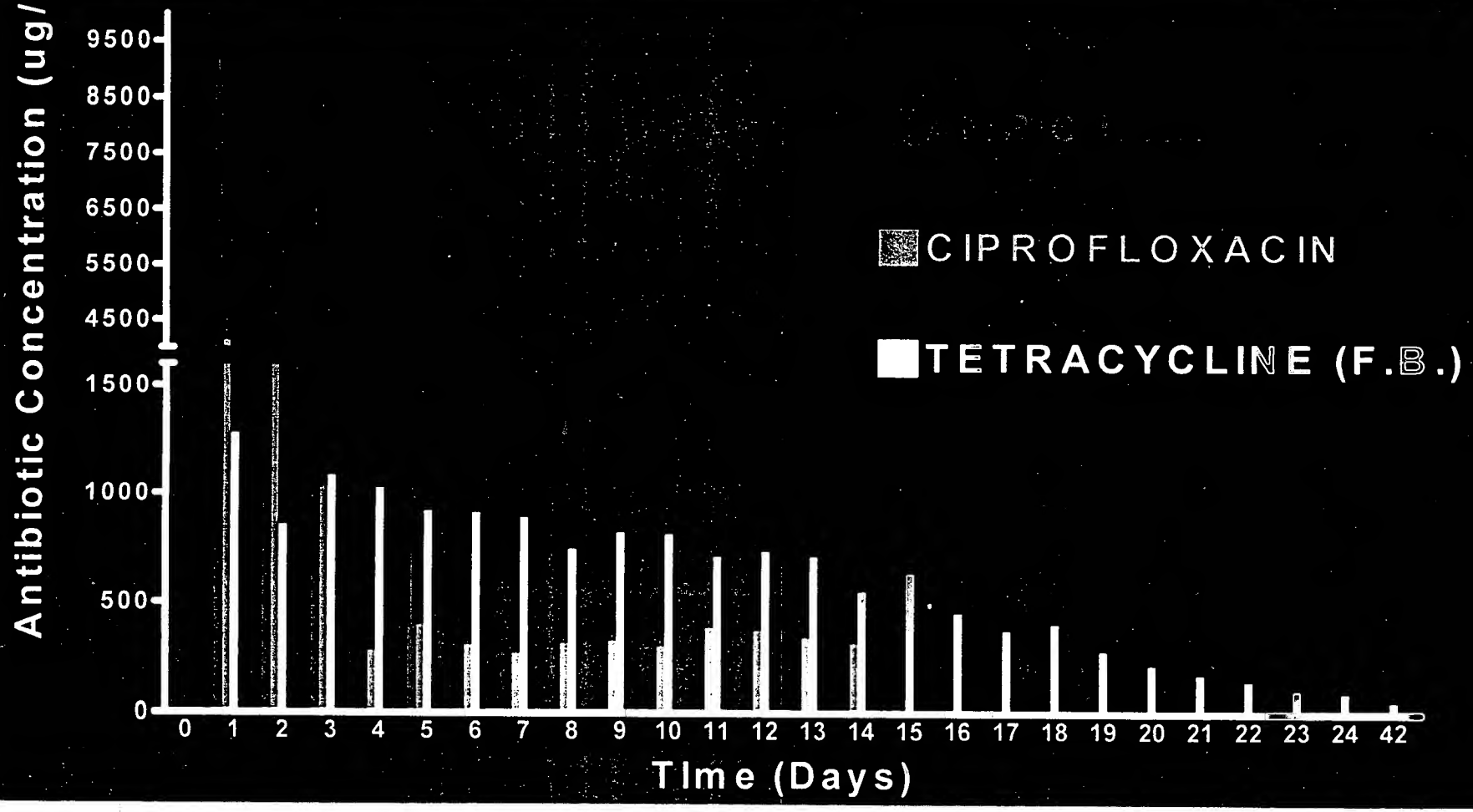
Long Term Delivery of Antibiotic is Accomplished by Loading It Above It's Solubility Limit in Fibrin Sealant



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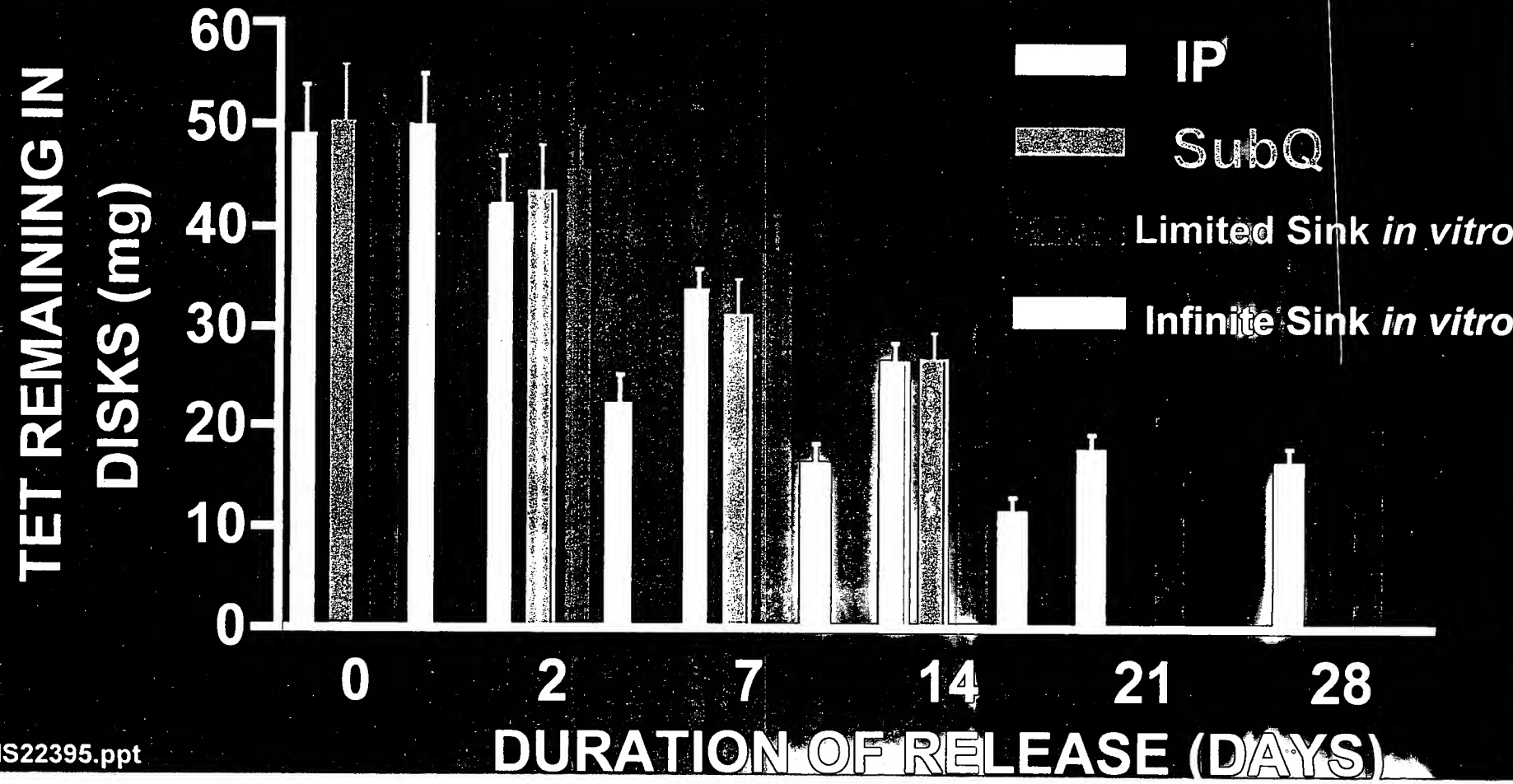
In Vivo Antibiotic Release Kinetics From Fibrin Sealant Match Limited-Sink *In Vitro* Delivery Kinetics



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Research and Development

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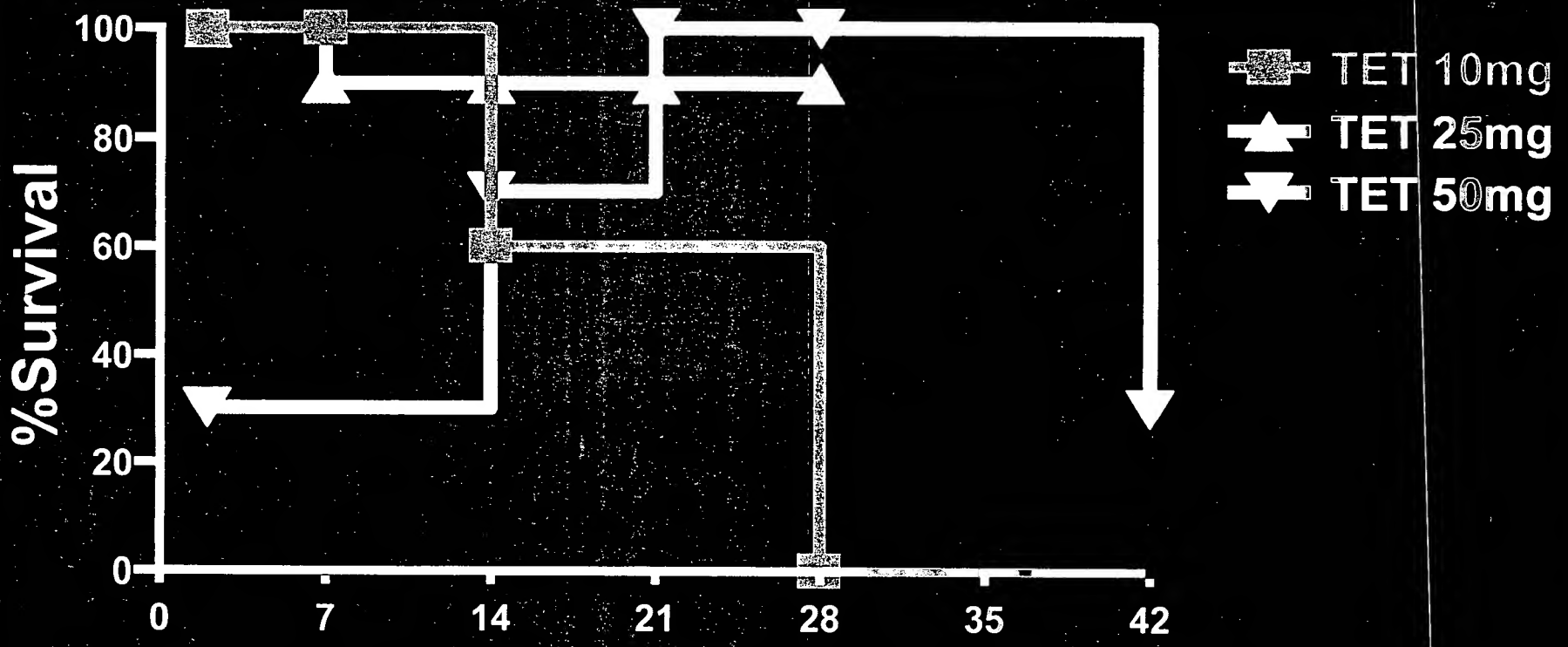
I.P. Implanted TET-Supplemented Fibrin Sealant Provides Extended Protection Against Lethal *S. aureus* Peritonitis



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